Achondrogenesis Type IB

Agenesis of Cartilage Interterritorial Matrix as the Link Between Gene Defect and Pathological Skeletal Phenotype

Alessandro Corsi, MD, PhD; Mara Riminucci, MD, PhD; Larry W. Fisher, PhD; Paolo Bianco, MD

● **Achondrogenesis type IB is a lethal osteochondrodysplasia caused by mutations in the diastrophic dysplasia sulfate transporter gene. How these mutations lead to the skeletal phenotype is not known. Histology of plastic-embedded skeletal fetal achondrogenesis type IB samples suggested that interterritorial epiphyseal cartilage matrix was selectively missing. Cartilage was organized in "chondrons" separated by cleft spaces; chondrocyte seriation, longitudinal septa, and, in turn, mineralized cartilaginous septa were absent. Agenesis of interterritorial matrix as the key histologic change was confirmed by immunohistology using specific markers of territorial and interterritorial matrix. Biglycan-enriched territorial matrix was preserved; decorin-enriched interterritorial areas were absent, although immunostaining was observed within chondrocytes. Thus, in achondrogenesis type IB: (1) a complex derangement in cartilage matrix assembly lies downstream of the deficient sulfate transporter activity; (2) the severely impaired decorin deposition participates in the changes in matrix organization with lack of development of normal interterritorial matrix; and (3) this change determines the lack of the necessary structural substrate for proper endochondral bone formation and explains the severe skeletal phenotype.**

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Achondrogenesis type I (ACG-I) is a rare and lethal skeletal dysplasia characterized by extreme limb shortening, marked discrepancy between head and trunk size, and severely delayed ossification. Two types, both inherited as autosomal recessive traits, have been recognized by radiology and histology.[1,2] By radiograph, type IA differs from type IB in the presence of rib fractures and the absence of ossification of vertebral pedicles. By histology, type IA is characterized by inclusion bodies in chondrocytes, and the cartilage matrix is close to normal; in type IB, the cartilage matrix is distinctly abnormal, showing rarefaction of the ground substance and a peculiar ringlike pericellular arrangement of collagen fibers.

Deficient sulfation of cartilage proteoglycans has been demonstrated in ACG-IB and has been related to a sulfate uptake defect caused by mutations in the diastrophic dysplasia sulfate transporter gene.[3–6] However, how these mutations lead to abnormal modeling and development of endochondrally formed bones is not known.

By means of histology and immunohistology, we investigated the morphopathogenesis of the skeletal abnormalities in a fetus with ACG-IB and demonstrated an abnormal organization of the epiphyseal cartilage due to the absence of an immunocytochemically recognizable interterritorial matrix. This abnormal pattern of cartilage matrix assembly explains well the abnormalities in endochondral ossification leading to shortness and deformity of the skeletal segments.

CLINICAL HISTORY

A white female fetus was the product of the third pregnancy of consanguineous (first cousins) parents; the mother was 25 years old and the father 34. Both parents were healthy. Two previous pregnancies had ended in spontaneous abortion. The present pregnancy was unremarkable. There was no history of familial dwarfism or known exposure to teratogenic drugs. Based on the ultrasonographic evidence of hydrops, extremely short limbs, and delayed ossification, the pregnancy was legally ended during the 18th week of gestation. Karyotype of the fetus was 46,XX.

At autopsy, the fetus was hydropic and showed a prominent cervical cystic hygroma. Weight was 250 g and crown–heel length 17 cm. External examination revealed large skull, short trunk with hypoplastic thorax and prominent abdomen, and severe shortness of the extremities. The face was flat and the nasal bridge slightly depressed. Fingers and toes were short and stubby. Internal examination revealed severely hypoplastic lungs. Cerebral, cardiovascular, gastrointestinal, and genitourinary abnormalities were not detected.

Radiographic study (Figure 1) showed severely delayed ossification. No ossification centers were evident for either the cervical or the last lumbar vertebrae. Iliac wings showed a crescent shape. The long bones of the limbs appeared as rudimentary ossicles. Scapulae were hypoplastic, and ribs were short and thin but not fractured. Mineralization of the cranial vault was slightly reduced. These findings were diagnostic of ACG-I. The absence of rib fractures and the evidence of ossification in the vertebral pedicles allowed the diagnosis of type IB.

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From the Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy (Drs Corsi and Riminucci); the Department of Experimental Medicine and Pathology, Section of Pathology, "La Sapienza" University, Rome, Italy (Drs Corsi, Riminucci, and Bianco); and the Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Md (Dr Fisher).

Reprints: Paolo Bianco, MD, Department of Experimental Medicine and Pathology, Section of Pathology, Università "La Sapienza," Policlinico Umberto I, Viale Regina Elena 324, 00161 Roma, Italy.

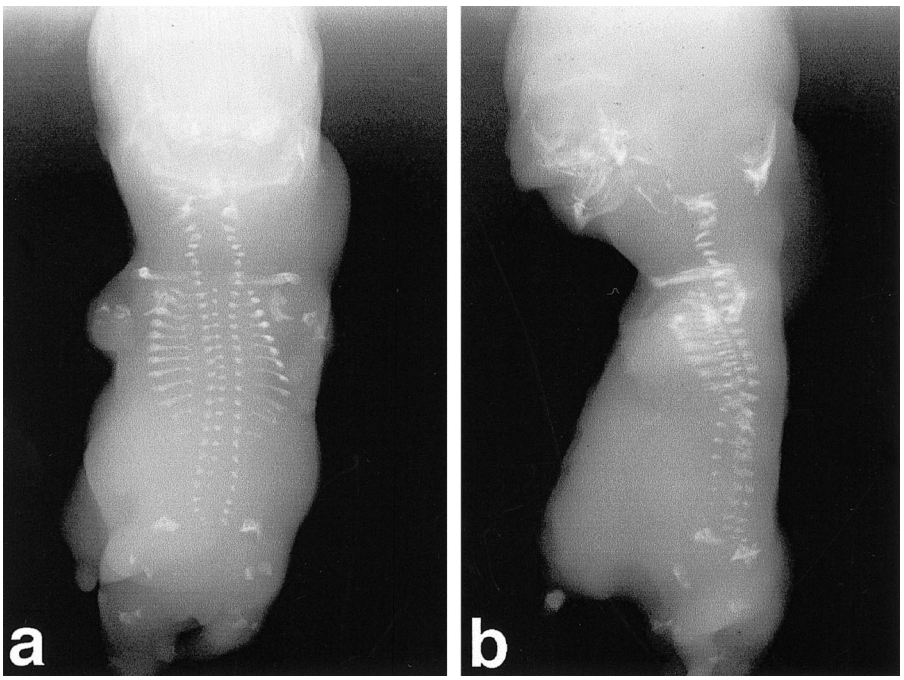

Figure 1. *Radiograph views of the fetus. Note the soft tissue shadow caused by the hydrops, delayed ossification, absence of rib fractures, crescent shape of the iliac wings, ossification of the vertebral pedicles, and the short and abnormally shaped long bones of the limbs.*

MATERIALS AND METHODS

Tissue Sampling and Histology

Ribs, long bones of the limbs, and parietal bones were dissected at the end of the autopsy and fixed in 4% formaldehyde (freshly made from paraformaldehyde) in 0.1 mol/L phosphate buffer, pH 7.2. Some of the samples were decalcified in buffered ethylenediaminetetraacetic acid and routinely embedded in paraffin; others were processed undecalcified for glycol-methacrylate embedding as described elsewhere.[7] Sections were stained with hematoxylin-eosin, May-Grünwald-Giemsa, periodic acid–Schiff, toluidine blue, silver-nitrate-methenamine, and Alcian blue.

Antibodies and Immunohistology

Two sequence-specific peptide antisera (LF-15 and LF-30) generated against synthetic peptides corresponding to amino acids 11–24 and 5–17 of the human core proteins of 2 small leucine-rich proteoglycans, biglycan and decorin, were used to characterize territorial or interterritorial cartilage matrices. In normal epiphyseal cartilage matrix, their distribution has been demonstrated to be mutually exclusive, even though both are expressed in epiphyseal cartilage chondrocytes.[8] Immunohistology was performed as described previously.[8]

RESULTS

Histology

Endochondral bone formation was notably abnormal (Figure 2). The cartilage-bone junction at the level of the growth plates was curved, with the concavity facing the cartilage; periosteal ossification projected beyond the metaphyseal margins in the form of bony spurs causing a cuplike appearance. Epiphyseal cartilages showed huge chondrocytes, frequently with intracytoplasmic vacuoles. At this level, the cartilage matrix showed a spongelike appearance in paraffin sections stained with hematoxylin-eosin. As shown by 3-μm-thick sections from plastic blocks, this feature appeared to be caused by the absence of a recognizable interterritorial matrix. The epiphyseal cartilage was composed of multiple discrete units of one or more chondrocytes encased in a territorial capsule and separated from each other by cleft spaces in which spindle fibroblast–like cells were easily recognizable. Overall, the breakdown of the usual matrix continuity of epiphyseal cartilage resulted in a mosaic of chondrocyte units (''chondrons''). At the growth plates, chondrocytes failed to align in columns, and no obvious longitudinal septa (which are normally derived from interterritorial areas) were detected; as a result, mineralization of the cartilage matrix (which normally occurs in longitudinal septa) was also absent. Histochemically, cartilage sites of positive staining with cationic dyes (toluidine blue and Alcian blue) and of staining for vic-glycolic groups (silver-nitrate-methenamine and periodic acid–Schiff) were limited to the matrix capsules around chondrocytes.

Immunohistology

The absence of a chemically identifiable interterritorial matrix was confirmed by the immunoreactivity of the epiphyseal cartilage for biglycan and decorin (Figure 3). In normal human fetal epiphyseal cartilage (Figure 3, a and b), territorial capsules of chondrocytes are enriched in biglycan but devoid of immunodetectable decorin, whereas the interterritorial matrix is enriched in decorin and appears devoid of biglycan.[8] In ACG-IB epiphyseal cartilage, biglycan immunoreactivity was found in the matrix encasing chondrocytes (Figure 3, c), consistent with a territorial matrix identity. In contrast, no decorin immunoreactivity was detected in the extracellular matrix (Figure 3, d). However, decorin labeling was observed in chondrocytes as well as in the spindle fibroblast–like cells permeating the cleft spaces between chondrons.

COMMENT

The clinicopathologic findings observed in our case accord well with the diagnosis of ACG-IB.[1,3–6,9,10] Even though it has been established that ACG-IB is caused by mutations in the diastrophic dysplasia sulfate transporter gene,[3–6] the mechanism by which these mutations lead to the severe bone phenotype has never, to our knowledge,

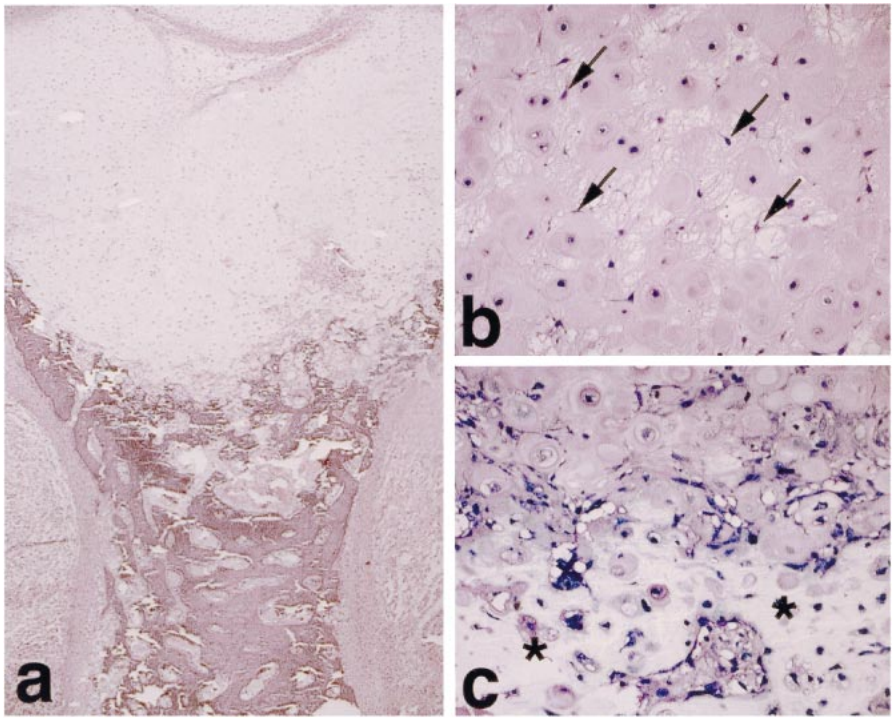

Figure 2. *a, Left femur, glycol-methacrylate embedding. Low-power magnification shows the abnormal cartilage-bone junction (hematoxylin-eosin, original magnification ×25). b, In the epiphyseal cartilage, matrix is condensed around chondrocytes, and spindle cells (arrows) permeate matrix clefts (May-Grünwald-Giemsa, original magnification ×140). c, At the growth plate, chondrocyte seriation and longitudinal septa are absent, and primary trabeculae (asterisks) do not contain cartilaginous cores (May-Grünwald-Giemsa, original magnification ×140).*

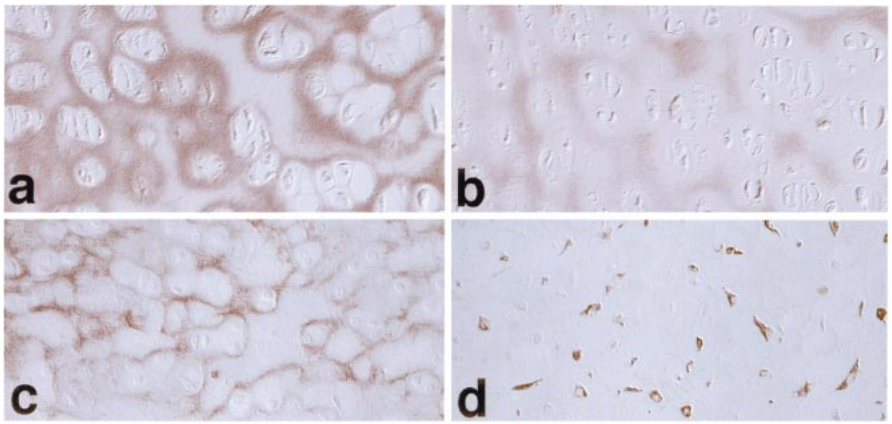

Figure 3. *Biglycan (a, c) and decorin (b, d) immunoreactivity in normal (a, b) and achondrogenesis type IB (ACG-IB) (c, d) epiphyseal cartilage. In ACG-IB, the cartilage matrix is immunoreactive for biglycan but not for decorin; chondrocytes and fibroblast-like spindle cells are both immunoreactive for decorin (immunoperoxidase, diaminobenzidine reaction, original magnification ×280).*

been investigated at tissue level. Our study disclosed a major change in the overall structural organization of the epiphyseal cartilage, which lies downstream of the gene defect and further signals the occurrence of a complex derangement in the supramolecular assembly of cartilage matrix. We observed significant abnormalities in epiphyseal cartilage matrix and, as a consequence, in endochondral bone formation. The cartilage matrix was markedly reduced and disclosed a ''chondron-mosaic'' appearance due to the absence of a recognizable interterritorial matrix. In the growth plates, chondrocyte seriation, longitudinal septa formation, and cartilage mineralization were absent, and the primary trabeculae did not contain an axial cartilaginous core. In addition, the clefts produced by the absence of the interterritorial matrix were diffusely permeated by spindle fibroblast–like cells, likely corresponding either to an ingrowth from the periosteum or the medullary cavity or to ''rest'' of the primitive mesenchymal cells in the cartilage anlage.

Previous studies have shown that biglycan and decorin are expressed in normal epiphyseal chondrocytes, but they are distributed in the cartilaginous matrix in a mutually exclusive pattern. The territorial matrix is enriched in biglycan but devoid of immunodetectable decorin; in contrast, the interterritorial matrix is enriched in decorin and devoid of biglycan.[8] Therefore, we have used biglycan and decorin as immunocytochemical markers of the territorial and interterritorial matrix, respectively. Consistent with the turning of the epiphyseal cartilage into a mosaic of discrete chondrons separated by clefts and fibroblast-like cells, immunohistology indicates the absence of a chemically recognizable decorin-enriched interterritorial matrix; in contrast, the biglycan-enriched territorial matrix was preserved. These data are consistent with a severe derangement in the rate of proteoglycan production and deposition resulting from mutations in the diastrophic dysplasia sulfate transporter gene. Of note, in spite of the lack of decorin immunoreactivity in the epiphyseal matrix, decorin core protein was immunodetectable within chon-

drocytes, indicating that the lack of its deposition is not caused by a lack of gene expression and protein synthesis.

Although mutations in the diastrophic dysplasia sulfate transporter gene are expected to result in complex and multiple chemical abnormalities of the cartilage matrix, the immunohistochemical distribution of biglycan and decorin discloses a major structural change in the overall organization of epiphyseal cartilage matrix, with important implications for the pathogenesis of the abnormal growth and modeling of endochondrally formed bones in ACG-IB. The lack of a properly assembled interterritorial matrix accounts for the production of clefts separating individual chondrons. The longitudinal septa separating columns of chondrocytes in the seriated zone of the growth plate represent the site of early mineralization and are formed from interterritorial matrix. Because longitudinal septa are not formed, mineralization cannot occur. As a consequence, the mineralized septa providing the scaffold for bone deposition cannot be formed, and osteogenesis itself is severely disturbed. The lack of proper assembly of the interterritorial matrix in the epiphyseal cartilage is therefore reflected in the severe impairment of multiple critical events that lie downstream along the sequence of growth plate maturation, vascular invasion, and bone formation.

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